Journal of Medicinal Chemistry
Brief Article

J. Med. Chem. **2009,** *52,* 7873–7877 **7873**
DOI: 10.1021/jm901131m

Design, Synthesis, and Biological Evaluation of Substituted Naphthalene Imides and Diimides as Anticancer Agent[∞]

Vincenzo Tumiatti,*[,†,§] Andrea Milelli,[†] Anna Minarini,[†] Marialuisa Micco,[†] Anna Gasperi Campani,[#,∥] Laura Roncuzzi,[#,⊥] Daniela Baiocchi,[#] Jessica Marinello,[‡] Giovanni Capranico,[‡] Maddalena Zini,[‡] Claudio Stefanelli,[‡] and Carlo Melchiorre[†]

[†]*Department of Pharmaceutical Sciences, Alma Mater Studiorum, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy,* [§]*Polo Scientifico-Didattico di Rimini, University of Bologna, 40100 Bologna, Italy,* [‡]*Department of Biochemistry "G. Moruzzi", University of Bologna, Via Irnerio 48, 40126 Bologna, Italy,* [#]*Department of Experimental Pathology, University of Bologna, Via S. Giacomo 14, 40126 Bologna, Italy,* [∥]*Interdepartmental Center for the Research on Cancer, Policlinico S. Orsola, University of Bologna, Via Massarenti 9, Bologna, Italy, and* [⊥]*Laboratory of Pathophysiology of Orthopaedic Implants, Istituto Ortopedico Rizzoli, Via di Barbiano 1/10, Bologna, Italy*

Received May 21, 2009

Naphthalimmide (NI) and 1,4,5,8-naphthalentetracarboxylic diimide (NDI) derivatives were synthesized and evaluated for their antiproliferative activity. NDI derivatives **1**−**9** were more cytotoxic than the corresponding NI derivatives **10**−**18**. The molecular mechanisms of **1** and **2** were investigated in comparison to mitonafide. They interacted with DNA, were not topoisomerase IIα poisons, triggered caspase activation, caused p53 protein accumulation, and down-regulated AKT survival. Furthermore, **1** and **2** caused a decrease of ERK1/2 and, unlike mitonafide, inhibited ERKs phosphorylation.

Introduction

The search for novel chemotherapeutic agents and approaches to cancer treatment is an active research field stimulated by the discovery of new biological targets and by the possibility of obtaining new drugs without serious and undesirable side effects.[1] Small molecules able to reversibly interact with the DNA structure represent a prolific research area for new potential anticancer agents.[2] Such molecules, known as intercalators, are typically characterized by a planar heterocyclic moiety of approximately the size and shape of a DNA base pair.[3] They insert perpendicularly into the DNA without forming any covalent bonds, and the formed complex is stabilized by hydrophobic, van der Waals, hydrogen bonding, and charge transfer forces. It has been demonstrated that these interactions may induce unwinding, lengthening, and stiffening of the double helix, introducing structural changes that can affect the cells' replication processes, leading to cellular death and genotoxic effects.[4] Several DNA binding modes were reported and include electrostatic interactions, groove binding, and threading intercalation.

It is well-known that, at physiological pH, protonated polyamines interact strongly with the phosphate residues of DNA.[5] Therefore, the inclusion of these basic functionalities on an intercalating moiety may improve the interaction with DNA structure by inserting their aromatic system between the base pairs, while the cationic heads bind with the major and minor grooves.[6] In the literature, there are several examples of naphthalimmide (NI[a]) and 1,4,5,8-naphtalentetracaboxylic diimide (NDI) derivatives as intercalating and anticancer agents.[2,7−10] Two important prototypes of NI series are mitonafide and amonafide (Figure 1) which, although characterized by a valuable anticancer profile, did not pass the phase II because of their toxicity.[11,12] Several attempts were performed to overcome this limitation by synthesizing different analogues.[13−15] Two examples of NDI series are N-DMPrNI and N-BDMPrNDI (Figure 1) which showed the ability to intercalate into steps containing at least one G:C base pair.[9]

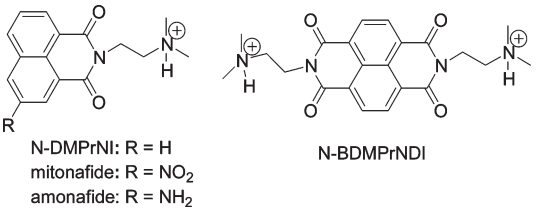


Figure 1. Chemical structures of N-DMPrNI and N-BDMPrNDI derivatives and reference compound mitonafide.

These considerations prompted us to design **1**−**18**, using NI and NDI scaffolds as molecular building blocks. **1**−**18** contain one or two basic polymethylene chains in their structures whose lengths may have relevance for the anticancer activity. The protonation of one or two basic nitrogen atoms at physiological pH strongly influences the DNA binding, as reported above.[6] To enhance the basicity of the terminal amine functions of lateral chains, a 2-methoxybenzyl substituent was chosen. This is because, as previously demonstrated,[16] this function improved the basicity when inserted on terminal nitrogen atoms of related polymethylene amines. It could also promote additional DNA hydrophobic interactions. Finally, the cytotoxic activity of the most interesting

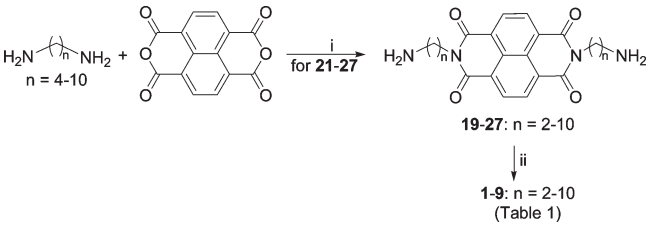
[∞] Dedicated to Professor Fulvio Gualtieri on the occasion of his retirement.

*To whom correspondence should be addressed. Address: Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy. Phone: +39-051-2099709. Fax: +39-051-2099734. E-mail: vincenzo.tumiatti@unibo.it.

[a] Abbreviations: NI, naphthalimmide; NDI, 1,4,5,8-naphthalenetetracarboxylic diimide, MAPK, mitogen-activated protein kinase; ERK, extracellular signal regulated kinase.

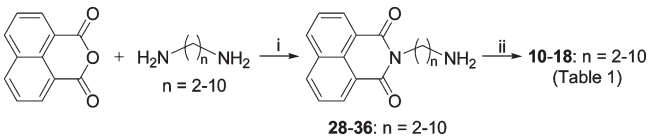
Published on Web 09/16/2009

Scheme 1[a]



[a] (i) EtOH−toluene 1:1, reflux, 24 h, quantitative yield; (ii) (a) 2-$MeOC_6H_4CHO$, toluene, reflux, 3 h; (b) $NaBH_4$, MeOH, room temp, 4 h, 21−70%.

Scheme 2[a]



[a] (i) EtOH, reflux, 24 h, 20−85%; (ii) (a) 2-$MeOC_6H_4CHO$, toluene, reflux, 3 h; (b) $NaBH_4$, EtOH, room temp, 3 h, 24−36%.

compounds, **1** and **2**, was investigated at the molecular level in comparison to mitonafide.[10]

Results and Discussion

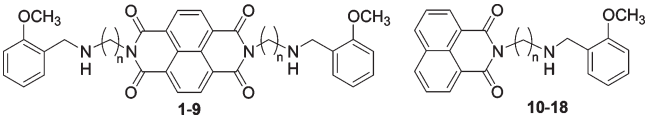
Chemistry. Compounds **1**−**9** were synthesized following the procedure reported in Scheme 1. Naphthalenetetracarboxylic dianhydride was condensed with the corresponding polymethylene diamine ($n = 4-10$) to give **21**−**27**. These compounds and conjugates **19** and **20**[17] were treated with 2-methoxybenzaldehyde followed by reduction with sodium borohydride of formed Schiff bases to afford the corresponding diamine diimides **1**−**9**. The same procedure was adopted for the synthesis of **10**−**18**, using benzo[*de*]isochromene-1, 3-dione as starting anhydride, through intermediate amines **28**−**36** (Scheme 2).

Growth-Inhibiting Activity. Derivatives **1**−**18** were evaluated by in vitro assays for their antiproliferative activity in human breast cancer (SKBR-3) and leukemia (CEM) cell lines. Growth inhibition induced by tested compounds was assessed by the 3-(4,5-dimethylthiazolyil-2)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were treated with the test compounds at concentrations ranging from 0.1 to 10 μM for 72 h.

The results shown in Table 1 reveal that bis-substituted derivatives are more potent than the corresponding monosubstituted derivatives, with the exception of **7** and **8** which showed comparable activity values with those of **16** and **17**. Interestingly, the chain length of the side chains of bis-substituted derivatives **1**−**9** significantly affected cytotoxic activity in both cell lines. The most potent were **2** and **9** with chain lengths of 3 and 10 methylene units, respectively, separating the nitrogen atoms of the side chains. This finding suggests that the terminal positive charged nitrogen atoms of **2** and **9** may interact with different anionic counterparts of DNA. The same pattern was not observed in the monosubstituted series, in which the only notable derivative was **17** with an IC_{50} on SKBR-3 of 0.5 μM, clearly indicating that a second lateral chain contributes to enhancing cytotoxic activity.

After these first screening assays, **1** and **2** were selected for further investigation to determine their cytotoxic molecular mechanism. Their choice was dictated by the observation that **2** was the most potent of both series of compounds on SKBR-3 and CEM cells, and **1** shares similar chemical features (side chain length) with mitonafide. Thus, the cytotoxic activity of **1** and **2** was further assessed against HL60 leukemia cells in comparison to mitonafide. **1** and **2** confirmed their antiproliferative activity toward this cell line, with a potency that was comparable to that of mitonafide, as revealed by their respective IC_{50} (μM) values of 0.9, 1.2, and 1.6 (Table 1).

Table 1. Cytotoxic Activity of **1**−**18** against SKBR-3 and CEM Cells after 72 h and against HL60 after 24 h of Compounds Exposure



		IC_{50} (μM)[b]		
compd[a]	*n*	SKBR-3	CEM	HL60[d]
mitonafide				1.6
1	2	0.7	1.3	0.9
2	3	0.2	0.4	1.2
3	4	0.7	0.8	nd[e]
4	5	1.1	1.1	nd[e]
5	6	1.2	2.2	nd[e]
6	7	1.7	2.5	nd[e]
7	8	1.0	1.6	nd[e]
8	9	0.4	1.4	nd[e]
9	10	0.2	0.7	nd[e]
10	2	2	na[c]	nd[e]
11	3	na[c]	na[c]	nd[e]
12	4	1.7	3.2	nd[e]
13	5	na[c]	na[c]	nd[e]
14	6	na[c]	na[c]	nd[e]
15	7	na[c]	na[c]	nd[e]
16	8	1.4	1.2	nd[e]
17	9	0.5	1.3	nd[e]
18	10	2	8	nd[e]

[a] **1**−**9**, di-*p*-toluensulfonate salts; **10**−**18**, *p*-toluensulfonate salts. [b] IC_{50} values represent the concentration causing 50% growth inhibition. They were determined by linear regression method. Each sample is the mean of six experiments. [c] Not active at 10 μM. [d] The IC_{50} values are reported as the mean of three determinations. Cell death was determined by dye exclusion. [e] Not determined.

DNA-Binding Properties. Mitonafide and NDI derivatives are well-known intercalators. To assess the ability of **1** and **2** to interact with DNA, DNA-binding studies were carried out in comparison to mitonafide, using a fluorometric intercalator displacement method.[18] DNA-binding activity was expressed as the drug concentration reducing by 50% the fluorescence of DNA-bound ethidium bromide. The EC_{50} is approximately inversely proportional to the binding constant, and an apparent binding constant[18] (K_{app}), which estimates the affinity of the drug for calf thymus DNA, can be calculated.[18,19] **1** and **2** turned out to potently bind DNA and displace ethidium bromide with EC_{50} values that were 2 orders of magnitude higher than that of the reference compound mitonafide (Table 2).

Topoisomerase Inhibition. Compounds **1**, **2**, **10**, and **17** were also evaluated by using the DNA SV40 portion as substrate for the recombinant human DNA topoisomerase IIα assays to verify whether they act as topoisomerase IIα poisons.[20] It turned out that they were ineffective in

Table 2. Binding to DNA by Ethidium Displacement Test of **1**, **2**, and Mitonafide

compd[a]	EC_{50} (nM)[a]	K_{app} (M^{-1})[b]
mitonafide	11500 ± 320	2.20×10^6
1	93 ± 4	2.72×10^8
2	122 ± 6	2.07×10^8

[a] EC_{50} values are defined as the drug concentrations that reduce the fluorescence of the DNA-bound ethidium by 50% and are reported as the mean of three determinations ± SEM. [b] Apparent binding constant (K_{app}) values have been calculated taking the ethidium binding constant as 10^7 M^{-1}.

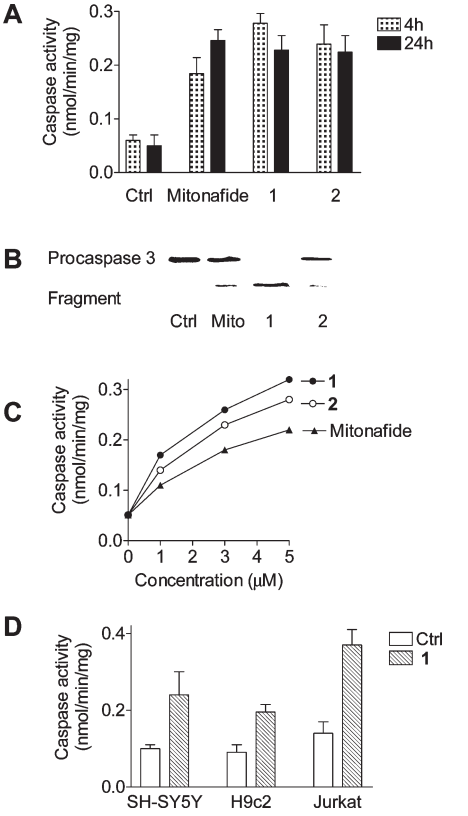


Figure 2. Derivatives **1** and **2** and mitonafide trigger caspase activation. (A) The activity of caspase proteases acting on the peptide sequence DEVD (DEVDase activity) was measured in HL60 cells treated for 4 or 24 h with 5 μM of the indicated compound. (B) HL60 cells were incubated for 4 h in the presence of the indicated compound (5 μM). Then the cellular proteins were extracted and analyzed by Western blotting. (C) HL60 cells were treated for 4 h with the indicated dose of the test compound. Afterward, cells were collected for caspase activity determination. (D) Caspase activation was measured in SH-SY5Y neuroblastoma, H9c2 heart cells, and Jurkat T-cells following treatment for 24 h with 5 μM **1**. Data are mean values ± SEM of triplicate measurements.

stimulating DNA cleavage by recombinant human topoisomerase IIα (Figure 1SI of Supporting Information).

Cytotoxic Effects. NI derivatives can exert their antiproliferative effect by multiple independent mechanisms.[14,15,21] First, we determined whether **1** and **2** caused the activation of apoptosis. To this end, HL60 cells were treated with **1**, **2**, and mitonafide, followed by the assessment of the activity of caspase proteases acting on the substrate sequence Asp-Glu-Val-Asp (DEVD), i.e., mainly effector caspases 3 and 7, whose activation represents a marker of apoptotic cell death.[22] It was found that **1** and **2**, like mitonafide, triggered caspase activation (Figure 2A). Western blotting analysis of

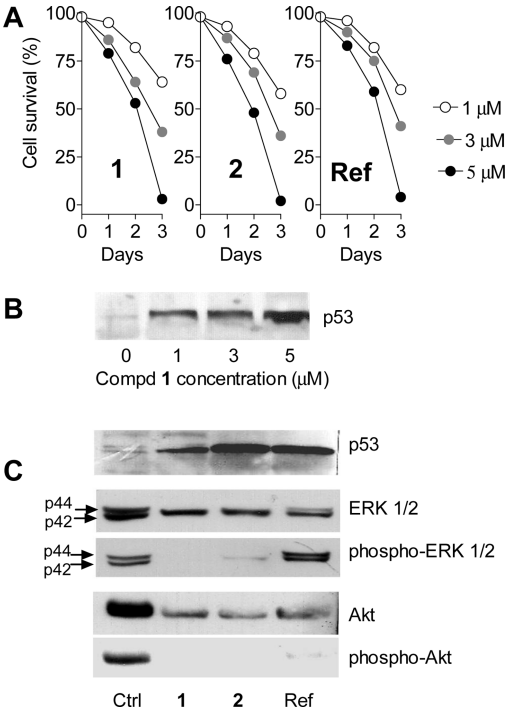


Figure 3. Effect of **1**, **2**, and mitonafide (Ref) on signal transduction pathway correlated to cell survival in SH-SY5Y neuroblastoma cells. (A) The cells were incubated for the indicated time in the presence of **1** and **2** or mitonafide at concentrations ranging from 1 to 5 μM. Then the number of alive viable cells was measured by dye exclusion. (B) The content of p53 protein was measured in extracts obtained from cells incubated 20 h in the presence of the indicated concentration of **1**. An amount of 50 μg of protein was separated and analyzed by Western blotting for each point. (C) The cells were incubated for 20 h in the presence of 5 μM of the indicated compound. Then cells were collected. The content and phosphorylation status of the indicated proteins in cell extracts were determined by Western blotting (50 μg of protein/lane).

protein extracted from cells treated with the aforementioned compounds confirmed the activation of caspase-3 (Figure 2B). In treated cells, we observed a reduction of the inactive precursor procaspase 3 (32 kDa) associated with the appearance of the proteolytic fragment, constitutive of the active enzyme (Figure 2B). It is worth noting that **1** and **2** triggered caspase activation rapidly. After a few hours of treatment, a large part of the procaspase 3 was processed into the active form, leading to apoptosis. The early activation of caspase activity increased in a dose-dependent manner associated with the increased number of cells committed to death (Figure 2C). We further investigated the ability of the most potent derivative **1** to trigger caspase activation on different cell lines, such as SH-SY5Y neuroblastoma cells, H9c2 heart cells, and Jurkat T-cells. Figure 2D shows that **1** elicited caspase activation in all tested cells. Activation of apoptosis can be initiated by extracellular apoptogenic compounds or intracellular insults and often requires the activation of specific signaling cascades.[23] The involvement of certain putative biochemical pathways in the induction of apoptosis was examined in SH-SY5Y neuroblastoma cells (Figure 3). These cells responded to **1** and **2** similarly to the other cell types previously evaluated, and 5 μM **1** or **2** caused a massive cell death after 72 h (Figure 3A). Mitonafide toxicity was very similar to that of **1** and **2**. Their IC_{50} values could be estimated in the range 1.4−1.9 μM. First, we examined whether the toxic effect of **1** and **2** was accompanied by

changes in the expression of the p53 protein, whose up-regulation is generally associated with DNA damage.[24,25] In cells treated for 20 h with the most potent derivative **1**, p53 accumulation was evident (Figure 3B) even with the lowest dose studied (1 μM), which caused a very limited toxicity at 24 h and about 60% of cell survival after 72 h. A large accumulation of the p53 protein was also evident following treatment with **2** or mitonafide (5 μM) for 20 h (Figure 3C). Next, the effect of **1** and **2** on signal transduction pathway involved in cell death and survival was investigated. In these studies, the cells were treated with 5 μM of the different agents for 20 h. At this time point, the amount of viable cells was quite high, ranging from 70% to 80%, but virtually all cells were committed to death within 72 h. The kinase Akt, an oncoprotein correlated to cell survival and proliferation, up-regulated in several cancers, and responsible for resistance to apoptotic cell death[26] which very recently has been associated with the toxic effect of an amonafide analogue, was examined.[21] Interestingly, **1**, **2**, and mitonafide caused an intense down-regulation of the kinase Akt and abolished its phosphorylation (Figure 3C). ERK1/2 mitogen-activated protein kinases are generally associated with cell growth, even if in some circumstances they may contribute to activation of apoptosis.[27] In the case of ERK1/2, the effect of **1** and **2** was completely different with respect to mitonafide (Figure 3C). In fact, mitonafide caused a slight decrease of p42 and p44 ERK proteins while increasing their phosphorylation. In contrast, **1** and **2** caused a large decrease of the p42 ERK2 protein and completely inhibited the phosphorylation of p42 and p44 ERKs, which are known to influence the survival of cancer cells.[28]

Conclusions

We synthesized several NI and NDI derivatives that possessed one or two basic functions, respectively, at the end of polymethylene chains, each characterized by a different length. It was demonstrated that cytotoxic activity is strongly influenced by the chain length and by the presence of NI and NDI scaffolds. The most potent compound, **2**, belongs to the NDI series and is endowed with a chain length of three methylene units. This compound, together with **1** (an NDI analogue of the reference compound mitonafide), showed the ability to bind DNA, trigger caspase activation, cause accumulation of p53 protein, and down-regulate the survival kinase AKT. However, **1** and **2** did not act as topoisomerase IIα poisons and caused a decrease of ERK1/2 and, unlike mitonafide, inhibited ERK's phosphorylation.

Finally, the potential toxicity profile, if any, of **1** and **2** should not be related to that of mitonafide because they have different chemical features and protein phosphorylation mechanisms.

Experimental Section

Melting points were taken in glass capillary tubes on a Buchi SMP-20 apparatus and are uncorrected. ESI-MS spectra were recorded on Perkin-Elmer 297 and WatersZQ 4000. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian VRX 200 and 300 instruments. The elemental analysis was performed with Perkin-Elmer 2400 CHN elemental analyzer. From all new compounds satisfactory elemental analyses were obtained, confirming > 95% purity.

General Procedure for the Synthesis of 1−18. A mixture of the appropriate diamine (**19**, **20**[17] or **21**−**27**) or amine (**28**−**36**) and 2-methoxybenzaldehyde (in a 1:2.4 or 1:1.2 molar ratio for **1**−**9** or **10**−**18**, respectively) in toluene was refluxed in a Dean−Stark apparatus for 3 h. Following solvent removal, the residue was taken up in EtOH, $NaBH_4$ (in a 1:5 or 1:2.5 molar ratio for **1**−**9** or **10**−**18**, respectively) was added, and the stirring was continued at room temperature for 4 h. The mixture was then made acidic with 6 N HCl and the solvent removed. Then the residue was dissolved in water and the resulting solution was washed with ether, made basic with K_2CO_3, and extracted with CH_2Cl_2 (3 × 30 mL). Removal of the dried solvent gave the desired products **1**−**18** that were converted into the corresponding *p*-toluenesulfonates salts (see Supporting Information for the characterization of compounds).

Acknowledgment. The authors thank Angela De Simone and Dorina Campana for technical assistance. This research was supported by a grant from MIUR, Rome (PRIN), University of Bologna (RFO), and Polo Scientifico-Didattico di Rimini.

Supporting Information Available: Experimental details for chemistry, biology, characterization, and elemental analyses of target compounds; experimental details and characterization of intermediate compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Chabner, B. A.; Roberts, T. G., Jr. Timeline: chemotherapy and the war on cancer. *Nat. Rev. Cancer* **2005**, *5*, 65–72.
(2) Martinez, R.; Chacon-Garcia, L. The search of DNA-intercalators as antitumoral drugs: what it worked and what did not work. *Curr. Med. Chem.* **2005**, *12*, 127–151.
(3) Persil, O.; Hud, N. V. Harnessing DNA intercalation. *Trends Biotechnol.* **2007**, *25*, 433–436.
(4) Ferguson, L. R.; Denny, W. A. Genotoxicity of non-covalent interactions: DNA intercalators. *Mutat. Res.* **2007**, *623*, 14–23.
(5) Schneider, H. J. Ligand binding to nucleic acids and proteins: Does selectivity increase with strength? *Eur. J. Med. Chem.* **2008**, *43*, 2307–2315.
(6) Strekowski, L.; Wilson, B. Noncovalent interactions with DNA: an overview. *Mutat. Res.* **2007**, *623*, 3–13.
(7) Yen, S. F.; Gabbay, E. J.; Wilson, W. D. Interaction of aromatic imides with deoxyribonucleic acid. Spectrophotometric and viscometric studies. *Biochemistry* **1982**, *21*, 2070–2076.
(8) Brana, M. F.; Ramos, A. Naphthalimides as anti-cancer agents: synthesis and biological activity. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 237–255.
(9) Liu, Z. R.; Hecker, K. H.; Rill, R. L. Selective DNA binding of (*N*-alkylamine)-substituted naphthalene imides and diimides to G+C-rich DNA. *J. Biomol. Struct. Dyn.* **1996**, *14*, 331–339.
(10) Ingrassia, L.; Lefranc, F.; Kiss, R.; Mijatovic, T. Naphthalimides and azonafides as promising anti-cancer agents. *Curr. Med. Chem.* **2009**, *16*, 1192–1213.
(11) Diaz-Rubio, E.; Martin, M.; Lopez-Vega, J. M.; Casado, A.; Benavides, A. Phase I study of mitonafide with a 3-day administration schedule: early interruption due to severe central nervous system toxicity. *Invest. New Drugs* **1994**, *12*, 277–281.
(12) Ratain, M. J.; Mick, R.; Berezin, F.; Janisch, L.; Schilsky, R. L.; Williams, S. F.; Smiddy, J. Paradoxical relationship between acetylator phenotype and amonafide toxicity. *Clin. Pharmacol. Ther.* **1991**, *50*, 573–579.
(13) Zhu, H.; Huang, M.; Yang, F.; Chen, Y.; Miao, Z. H.; Qian, X. H.; Xu, Y. F.; Qin, Y. X.; Luo, H. B.; Shen, X.; Geng, M. Y.; Cai, Y. J.; Ding, J. R16, a novel amonafide analogue, induces apoptosis and G2-M arrest via poisoning topoisomerase II. *Mol. Cancer Ther.* **2007**, *6*, 484–495.
(14) Van Quaquebeke, E.; Mahieu, T.; Dumont, P.; Dewelle, J.; Ribaucour, F.; Simon, G.; Sauvage, S.; Gaussin, J. F.; Tuti, J.; El Yazidi, M.; Van Vynckt, F.; Mijatovic, T.; Lefranc, F.; Darro, F.; Kiss, R. 2,2,2-Trichloro-*N*-({2-[2-(dimethylamino)ethyl]-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin- 5-yl}carbamoyl)acetamide (UNBS3157), a novel nonhematotoxic naphthalimide derivative with potent antitumor activity. *J. Med. Chem.* **2007**, *50*, 4122–4134.
(15) Mijatovic, T.; Mahieu, T.; De Neve, N.; Dewelle, J.; Simon, G.; Dehoux, M. J. M.; van der Aar, E.; Haibe-Kains, B.; Bontempi, G.; Deacestecker, C.; Van Quaquebeke, E.; Darro, F.; Kiss, R. UNBS5162, a novel naphthalimide that decreases CXCL

chemokine expression in experimental prostate cancers. *Neoplasia* **2008**, *10*, 573–586.
(16) Tumiatti, V.; Rosini, M.; Bartolini, M.; Cavalli, A.; Marucci, G.; Andrisano, V.; Angeli, P.; Banzi, R.; Minarini, A.; Recanatini, M.; Melchiorre, C. Structure−activity relationships of acetylcholinesterase noncovalent inhibitors based on a polyamine backbone. 2. Role of the substituents on the phenyl ring and nitrogen atoms of caproctamine. *J. Med. Chem.* **2003**, *46*, 954–966.
(17) Licchelli, M.; Linati, L.; Orbelli Biroli, A.; Perani, E.; Poggi, A.; Sacchi, D. Metal-induced assembling/disassembling of fluorescent naphthalenediimide derivatives signalled by excimer emission. *Chemistry* **2002**, *8*, 5161–5169.
(18) Morgan, A. R.; Lee, J. S.; Pulleyblank, D. E.; Murray, N. L.; Evans, D. H. Review: ethidium fluorescence assays. Part 1. Physicochemical studies. *Nucleic Acids Res.* **1979**, *7*, 547–569.
(19) McConnaughie, A. W.; Jenkins, T. C. Novel acridine−triazenes as prototype combilexins: synthesis, DNA binding, and biological activity. *J. Med. Chem.* **1995**, *38*, 3488–3501.
(20) De Isabella, P.; Zunino, F.; Capranico, G. Base sequence determinants of amonafide stimulation of topoisomerase II DNA cleavage. *Nucleic Acids Res.* **1995**, *23*, 223–229.
(21) Wang, J.; Wu, A.; Xu, Y.; Liu, J.; Qian, X. M(2)-A induces apoptosis and G(2)-M arrest via inhibiting PI3K/Akt pathway in HL60 cells. *Cancer Lett.* **2009**, *283*, 193–202.
(22) Lavrik, I. N.; Golks, A.; Krammer, P. H. Caspases: pharmacological manipulation of cell death. *J. Clin. Invest.* **2005**, *115*, 2665–2672.
(23) Cross, T. G.; Scheel-Toellner, D.; Henriquez, N. V.; Deacon, E.; Salmon, M.; Lord, J. M. Serine/threonine protein kinases and apoptosis. *Exp. Cell Res.* **2000**, *256*, 34–41.
(24) Lakin, N. D.; Jackson, S. P. Regulation of p53 in response to DNA damage. *Oncogene* **1999**, *18*, 7644–7655.
(25) Vogelstein, B.; Lane, D.; Levine, A. J. Surfing the p53 network. *Nature* **2000**, *408*, 307–310.
(26) Manning, B. D.; Cantley, L. C. AKT/PKB signaling: navigating downstream. *Cell* **2007**, *129*, 1261–1274.
(27) Shaul, Y. D.; Seger, R. The MEK/ERK cascade: from signaling specificity to diverse functions. *Biochim. Biophys. Acta* **2007**, *1773*, 1213–1226.
(28) Balmanno, K.; Cook, S. J. Tumour cell survival signalling by the ERK1/2 pathway. *Cell Death Differ.* **2009**, *16*, 368–377.